



ISSN: 0975-833X

RESEARCH ARTICLE

GAP MEASUREMENT OF THREE DIFFERENT ADHESIVE SYSTEMS WITH TWO DIFFERENT RESIN COMPOSITES- A CONFOCAL MICROSCOPIC STUDY

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ARTICLE INFO

Article History:

Received 20th September, 2012

Received in revised form

29th October, 2012

Accepted 25th November, 2012

Published online 18th December, 2012

Key words:

Gapwidth,
Self-etching,
Class V,
Bonding agent.

ABSTRACT

Objective: Marginal gap formation around composite resin restorations is responsible for fluid penetration, marginal discoloration, and eventually clinical failure of the restoration. The purpose of this study was to evaluate a range of contemporary adhesive systems to determine the marginal gap width developed at the resin-tooth interface in Class V cavity margins with a Confocal Laser Scanning Microscope.

Materials and Method: Thirty-six caries-free freshly extracted premolar teeth were selected and class V buccal cavities were prepared. The teeth were randomly assigned into three groups of 12 teeth each [Group I-Prime & Bond NT, Group II-AdheSE, Group III-Xenobond], which were further subdivided into 2 subgroups. All teeth belonging to subgroup "A" were restored with Durafil resin composite and subgroup "B" with Solitaire resin composite. The teeth were then sectioned longitudinally and were evaluated under CLSM.

Results: Statistical analysis was done using Student independent T-test and one-way analysis of variance (ANOVA). Comparing among the groups, mean marginal gap width is found to be maximum in Group III followed by Group II and Group I respectively.

Conclusion: Least marginal gapwidth formation is seen in single bottle adhesive system, Group IB (Prime & Bond NT).

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INTRODUCTION

The longevity of conventional class V resin composite restoration is relatively short. It has been attributed to gap formation around restorations due to resin shrinkage during polymerization or poor adhesion of the resin to the walls of the cavity.^[1,2] The gap formation between the restoration and the tooth structure is thus responsible for bacteria and fluid penetration, marginal discoloration, and consequently clinical failure.^[3] Several factors, such as polymer matrix composition, filler size, filler particle type and degree of conversion are responsible for the behavior of the resin composite. This research continues in an effort to develop an effective dentin bonding agent for bonding resin composite to dentin in order to retain restorations and to seal margins located in dentin and/or cementum.^[4] Similarly resin composite development has also moved from the era of macrofilled to microfilled, hybrids to microhybrids, and recently packable and nanocomposite have been developed to overcome the drawbacks.

The purpose of this study was to evaluate the marginal adaptation of mixed class V composite restoration with three different types of adhesive systems with Confocal Laser Scanning Microscope (CLSM). The advantage of using CLSM is that it enables direct non-invasive serial optical sectioning of intact and even living specimens.

MATERIALS AND METHOD

Thirty-six caries-free freshly extracted premolar teeth were selected for the study. They were cleaned ultrasonically and stored in normal saline. Class V buccal cavities were prepared in each tooth with the following specifications: depth 2.5mm, occlusogingival height 2mm, and mesiodistally 3mm. The teeth were randomly assigned into three groups of 12 teeth each [Group I-Prime & Bond NT, Group II-AdheSE, Group III-Xenobond], which were further subdivided into 2 subgroups (A & B), thus making a total of 6 subgroups, n = 6 teeth representing each subgroup. All teeth belonging to subgroup "A" were restored with Durafil resin composite and subgroup "B" with Solitaire resin composite. The composition of the adhesive systems used is given in Table 1. The adhesive systems were applied according to the manufacturer's instructions.

The resin composite restorations were polymerized incrementally using a visible light curing unit (Astralis 3) for 40 seconds at a distance of 2mm with intensity of 400mW/cm². Excess resin composite was removed with a finishing bur and the specimens were stored in normal saline for 24 hours. Following storage, the teeth were painted with nail varnish to within 2 mm of the cavity margin and were then immersed in Rhodamine B, (Hichem lab, Batch no: 0496/496/240372, Bombay, India), laser dye for a period of 72 hours, which emits fluorescence when excited with green light

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of 543 nm. The teeth were then sectioned longitudinally using diamond disk with copious amount of water coolant. Marginal gap width was measured using Confocal Laser Scanning Microscope (Zeiss LSM 510) at 3 different sites (Fig I):

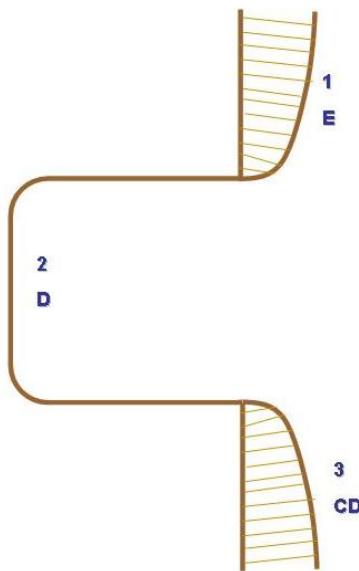


Fig. I: A Schematic diagram of margin measurement sites: Enamel (E), Dentine (D), and Cementum or Dentine (CD).

1. 1 mm from enamel junction.
2. Pulpal wall.
3. 1 mm from gingival margin on cementum or dentin.

The optical sections were taken at 1 μm interval starting from 30 μm and the images were then analyzed using LSM 510 software.

RESULTS

The marginal gap width of three adhesive systems at three different sites was calculated. The comparative Mean, Standard Deviation and Test of Significance of mean values between different adhesive systems of Subgroup A and Subgroup B at three different sites are given in Table II. Statistical analysis was done to compare values within and among the groups using Student independent T-test and one-way analysis of variance (ANOVA). Multiple range test by Tukey H.S.D (Honestly significant difference) procedure was employed to identify the significant group at 5% level. In the present study $P<0.05$ was considered as the level of significance.

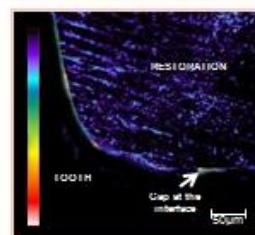
Comparing among the groups

- Mean marginal gap width is found to be maximum in Group III followed by Group II and Group I respectively.
- Comparing among the three sites studied, site 3 showed maximum mean value followed by site 1 and site 2 respectively.

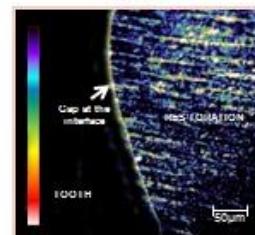
When comparing the overall results

Group I subgroup B showed superior results.

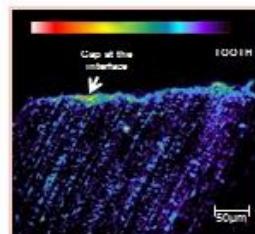
GROUP IA (PRIME AND BOND NT)



Site 1

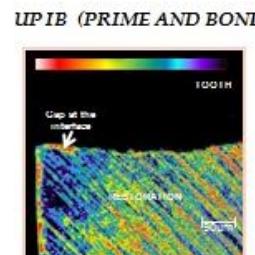


Site 2

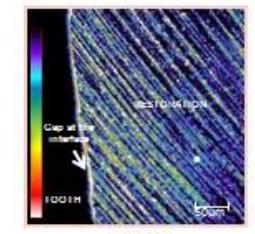


Site 3

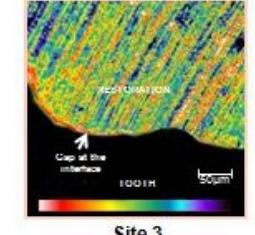
Fig II: Group IA (Prime Bond NT)



Site 1



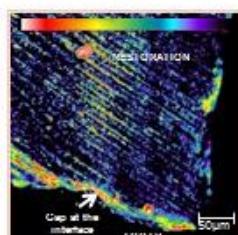
Site 2



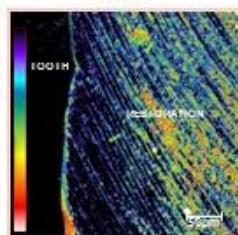
Site 3

Fig III: Group IB (Prime Bond NT)

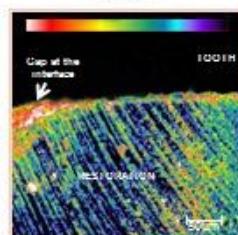
GROUP IIA (AdheSE)



Site 1



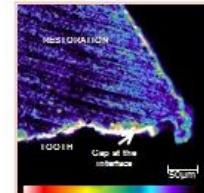
Site 2



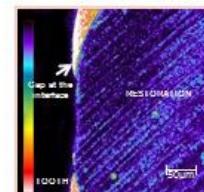
Site 3

Fig IV: Group IIA (AdheSE)

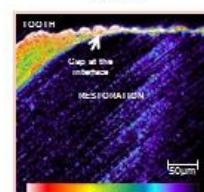
GROUP IIIA (Xeno III)



Site 1



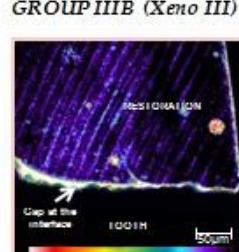
Site 2



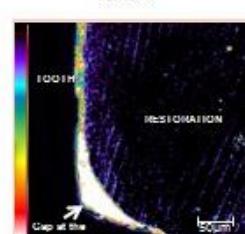
Site 3

Fig VI1: Group IIIA (Xeno III)

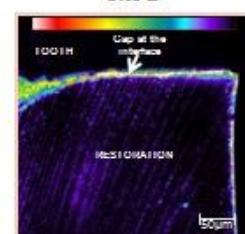
GROUP IIIB (Xeno III)



Site 1



Site 2



Site 3

Fig V: Group IIIB (AdheSE)

Fig VII1: Group IIIB (Xeno III)

Table I: Composition of the Adhesive Systems

GROUP I			
Self priming adhesive system		<ul style="list-style-type: none"> ✓ Di- and trimethacrylate resins ✓ Nanofillers – Amorphous silicon dioxide ✓ PENTA ✓ Photoinitiators ✓ Stabilizers ✓ Cetylamine Hydrofluoride ✓ Acetone 	
Prime-Bond NT			
(Dentsply, 0205000335)			
GROUP II		Primer	
Self-etching adhesive system		<ul style="list-style-type: none"> ✓ Dimethacrylate ✓ Phosphonic acid ✓ Initiators ✓ Stabilizers ✓ Water 	
AdheSE (Vivadent, F 25882)		Adhesive	
		<ul style="list-style-type: none"> ✓ HEMA ✓ Dimethacrylate ✓ Silicon dioxide ✓ Initiators ✓ Stabilizers 	
GROUP III		Liquid A:	
Single step self-etching adhesive system		<ul style="list-style-type: none"> ✓ HEMA, Alcohol, Purified water, Butylated hydroxy modified Toluene (BHT), and highly dispersed silicon dioxide. 	
Xeno Bond (Dentsply, 0303001361)		Liquid B:	
		<ul style="list-style-type: none"> ✓ Phosphoric acid modified methacrylate resins, Monofluoro phosphazene, polymethacrylate resin, Urethane dimethacrylate, BHT, Camphorquinone and Ethyl-4-dimethyl aminobenzote. 	

Table II : Comparison of Mean values between Subgroup A and Subgroup B at three different sites

	Group I		P value	Group II		P value	Group III		P value
	IA (μm)	IB (μm)		IIA (μm)	IIIB (μm)		III A (μm)	III B (μm)	
Site 1	5.69 \pm 0.60	2.46 \pm 0.48	< 0.0001 (Sig)	13.60 \pm 3.39	5.16 \pm 0.58	< 0.002 (Sig)	46.05 \pm 3.77	38.56 \pm 5.25	< 0.0001 (Sig)
Site 2	0.34 \pm 0.84	0.0 \pm 0.0	< 0.0001 (Sig)	9.31 \pm 1.72	0.38 \pm 0.92	< 0.0001 (Sig)	45.14 \pm 14.66	15.64 \pm 0.84	< 0.0001 (Sig)
Site 3	11.87 \pm 1.02	3.74 \pm 0.81	< 0.0001 (Sig)	23.05 \pm 2.06	10.45 \pm 0.81	< 0.0001 (Sig)	52.70 \pm 8.05	50.12 \pm 5.49	< 0.0001 (Sig)

DISCUSSION

The concept of restorative dentistry has been continually changing during the last four decades and adhesive dentistry has steadily gained importance. In the last decade, there has been a widespread increase in the use of composite material for restorations providing strength, esthetics, and durability. However, these polymeric material and their respective inorganic fillers were not particularly adhesive to enamel or dentin and thus there arose a need for suitable adhesion between the restorative material and tooth structure.^[4,5] However, these polymeric material and their respective inorganic fillers were not particularly adhesive to enamel or dentin and thus there arose a need for suitable adhesion between the restorative material and tooth structure. Many clinical trials exist with class V resin composite restorations, but according to Harris et al none of them offers evidence of complete marginal sealing.^[5] Various factors involved in the deficiency of marginal sealing are: polymerization shrinkage, adhesion to the cavity walls, viscosity and stiffness of the composite resin and flexibility of the cavity walls. The contraction stress in composite also, plays an important role in marginal adaptation. The contraction stress depends upon the type and level of fillers included. The overall polymerization shrinkage depends on the amount of polymer matrix present but an increase in filler level results in reduced polymerization

shrinkage, according Munksgaars and Iga et al. On the other hand, the stiffness of the composite is also increased at higher filler level. Thus, the composite stiffness and amount of contraction both plays an important role in generation of stress in composite restorations. Therefore, protocols for measuring marginal gap formation were developed to evaluate the marginal adaptation of resin composite restoration in Class V cavity. ^[6,7] The use of confocal microscopes over other, various methods of assessing the seal of dental restorative materials, was suggested because it enables thin optical sections to be made below the surfaces of intact specimens. This type of microscopy is thus ideally suited for investigation of the penetration, fit and thickness of adhesive bonding agents used in dental restorations.

In this in vitro study, Group I (Prime & Bond NT) showed the least gap formation. Prime & Bond NT is one application nanofilled bonding agent, which contains PENTA, a self-etching agent and with a low pH. The molecule of PENTA provides a better infiltration into the tooth structure. Di and trimethacrylate resins are elastomeric resins added to it to form a combination of relatively rigid and flexible molecule. Amorphous silica is a nanofiller (0.0007 μm), which has been functionalized by a special sialanization process making the filler more compatible with the resin matrix and allows it to serve as a cross linker. Cetylamine hydrofluoride provides the

release of the fluoride ions. Acetone allows excellent wetting of the conditioned dentin surfaces and acts as a water chaser, which helps in the diffusion of primer into the wet dentin substrates.^[8,9] Prime & Bond NT is an acetone based adhesive. Jacobsen et al reported that acetone is a superior solvent for hydrophilic primer better than water. It acts as a water chaser and helps in diffusion of the primer into the dentin substrate. This diffusion helps in providing a better bond strength. The nanofillers accumulated at the top of the hybrid layer, formed a palisade and clearly penetrate into the dentinal tubules.^[9] The least gap formation may be due to the fact that filled adhesives are designed to provide stress relief between tooth and the restorative material, which helps to preserve the marginal integrity. Another advantage of filled adhesives is that the film is thick enough to eliminate the problem of inhibition by oxygen. This can be correlated with the previous studies, which stated that the presence of an adequate thickness of adhesive resin is an important factor in developing a secure bond. Group II (AdheSE) showed less gap formation than Group III (Xeno Bond) but was statistically much lower when compared to Group I. It could probably be attributed to the fact that the primer is not rinsed after the application but air dried only.

The calcium and phosphate ions that were dissolved from hydroxyapatite crystals must have been suspended in the watery solutions of the primer. When the water is evaporated during air drying, the concentrations of solubilized calcium and phosphate ions within the primer may exceed the solubility constant for a number of calcium phosphate salts. Presumably minerals will then precipitate within the primer. These high concentration of calcium phosphate will tend to limit further dissolution of apatite due to common ion effects of calcium and phosphate and thereby limit the depth of surface demineralization. On the other hand it is very likely that the binding of calcium ions to the phosphate residues in the primer molecules contributes to the inactivation of molecules acidity (early buffering). It was thought that even the higher dissolution constant (pKa value) and the molecular weight of this acidic monomer contribute to this process.^[10,11] Group III (Xeno Bond) showed the maximum gap formation. The contents of liquid 'A' and liquid 'B' of Xeno III is mixed just before the application. The water in liquid A reacts with the acidic monomer and breaks the basic bonds to make it more reactive. Controlling this reaction after mixing becomes difficult and probably this could have been the reason for maximum gap formation.

Moreover, the evaporation of solvents is restricted and they may block the penetration of the monomer. The etching pattern of a self-etching adhesives is not as deep as that with phosphoric acid etching according to a study by Tay et al. But it performs a kind of 'nanoretention' with the superficial layer compared with etched enamel.^[12,13] These nanoretentions might produce the same bond strength as with conventional etching with phosphoric acid gel. However, this has been entirely supported from a scientific standpoint and cannot be generalized because it depends on enamel structure (ground/unground), cutting direction of the enamel prisms, the class of cavity involved and the product itself and the operator too. An alternative possibility for the microgap formation could be due to weak adaptation of the resin composite to the oxygen inhibited layer of the bonding agent.^[14] The higher

hydrophilia of self-etching adhesives – a phenomenon that initiated hydrolysis of resin polymers and even leaching of monomeric or oligomeric resin components from the resin-dentin interfaces, might lead to a kind of marginal staining. This occurrence is not based on the mode of demineralization (acidic monomers vs phosphoric acid) but is mainly correlated to the hydrophilia of the self-etching adhesives, which contain more hydrophilic monomers than conventional adhesives.

From the above results it has been shown that subgroup A showed increased gap formation when compared to subgroup B (Table II). Previous studies have shown that an inverse relationship exists between inorganic filler loading and monomer conversion. This could primarily be explained by the fact that lower the volume of filler, higher the volume of resin matrix. Since fillers are solids and do not shrink it is the fluid resin matrix phase that shrinks and results in physical deformations. Thus more resin results in greater physical deformation and larger shrinkage from one with more filler and less resin, resulting in less shrinkage.^[14,15] Thus the amount of polymerization shrinkage is related to the extent of polymerization reaction, filler size, filler loading, amount of monomer, type of monomer and degree of cure. Hence the filler content in the resin matrix plays a vital role in polymerization shrinkage.^[15] When comparing among the three different sites it has also been shown that site 3 showed more gap formation than site 1. This can be attributed to the fact that gingival margin contains higher inorganic content, variations in tubular structure, and presence of outward fluid movement. Another possibility is that the presence of enamel on one side of the restoration may be responsible for an imbalance i.e. possible increase in the gap formation on the dentin side, since enamel/composite bond is higher than the dentin/composite bond according to Davidson and De Gee et al. Thus, the complex morphology of class V defects with margins partly in enamel as well as in root dentin presents a challenging task for the restorative material.^[16,8]

The behavior of self-etching materials on enamel and dentin has been a controversial subject. Some studies have shown that self-etching systems perform well on enamel and dentin *in vitro* whereas others report insufficient bonding results.^[17,18] Interestingly it has been shown that no direct relationship exists between the bond strength of the materials and their sealing ability.^[19,20] Thus the adhesive system with good bonding strength cannot be documented to have good marginal sealing ability. Further studies with regard to bond strength, hybrid layer formation has to be carried out. Hence the search of simple, strong, and reliable dental adhesives is continuing to exercise the minds of dental researchers and scientists. Considering the drawbacks of self-etching primer obtained from this *in vitro* study, its clinical usage has to be restricted unless and otherwise indicated. Within the limitations of this *in vitro* study, it was concluded that,

- None of the tested bonding systems guaranteed a perfect marginal seal in dentin as well as in enamel. Despite improvements in the formulation, marginal quality, and sealing ability of adhesive systems to dentin is still inferior with enamel margins.
- Least marginal gap width formation is seen in single bottle adhesive system, Group IB (Prime & Bond NT).

- The use of self-etching primer should be restricted until further research validates its use universally.

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